Exhibit 36

Root morphological effects on Mg uptake in five tall fescue lines

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Key words *Festuca arundinacea* Magnesium nutrition Nutrient concentration Root diameter Xylem diameter

Summary A greenhouse experiment was conducted with tall fescue (*Festuca arundinacea* Schreb.) lines to determine the influence of root diameter and Mg concentration in nutrient solution on Mg uptake into shoots and roots. Propagules of 4 clonal tall fescue lines differing in root morphology, and a selection of 'Kentucky 31' (Ky 31), were grown for 39 or 70 days in 12-liter tanks containing a complete nutrient solution and Mg concentrations of 3, 21, 42, 125, 250, and 500 μM as $MgSO_4$. Root diameters averaged 0.98 mm for line AU-7; 0.83 mm for line AU-264; 0.72 mm for line AU-718; 0.72 mm for line AU-5; and 0.69 mm for Ky 31. At 39 days, leaf Mg concentration increased from about 1200 μg/g at the 3 μM concentration to about 2200 to 2400 μg/g at the 125 μM Mg concentration. Consistently, the large diameter root (LDR) lines AU-7 and AU-264 contained less Mg than the small diameter root (SDR) lines AU-5 and AU-718 and the selection of Ky 31 at both 39 and 70 days. Root Mg concentration was 50% of leaf Mg concentration. An Eadie-Hofstee plot indicated that influx of Mg proceeds via dual uptake mechanisms. The influx mechanism of the tall fescue line AU-7 appears to be saturated at a lower Mg concentration than the other fescue lines. SDR lines AU-5 and AU-718 have a larger capacity to accumulate Mg from solution.

Introduction

Consideration of root/soil interactions is essential in adapting tall fescue (*Festuca arundinacea* Schreb.) to the soil and climatic conditions of the Coastal Plain region of the Southeastern United States. Most of these soils are highly susceptible to formation of compaction layers, or plowpans[5]. The limitation of root growth due to compaction layers has been cited as a causal factor of drought stress and resulting stand decline which is characteristic of tall fescue in that area[2].

Williams *et al.*[16] demonstrated that morphological differences in roots exist between tall fescue lines, and that these differences are associated with drought resistance due to differential penetration of plowpans. A line with large diameter roots (LDR) was able to penetrate the plowpan to access subsoil water and survive severe drought stress better than a small diameter root (SDR) line which was restricted to the top 25 cm of soil.

The effects of differences in root morphology among tall fescue lines are not limited to differential abilities to penetrate compaction layers. Elkins *et al.*[4] showed a differential effect of nematode infection on Mg

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uptake in SDR and LDR tall fescue. The ability of LDR fescue to penetrate deeply into the soil reduced susceptibility to injury from plant-parasitic nematodes because less of the root system grew in nematode-infested soil[4]. Torbert *et al.*[15] showed differences in soil solution concentrations of NO_3–N under LDR and SDR tall fescue. However, they concluded that monitoring soil solution concentrations of K, Mg, and Ca in the field was not an adequate indicator of differences in tall fescue root activity.

Much effort has been expended in selecting tall fescue lines that will accumulate adequate Mg to prevent hypomagnesemic tetany in ruminant animals[8,10,12,13,14]. Magnesium accumulation in tall fescue lines requires a large volume of roots without suberized endodermis[3]. Fescue root anatomy (diameter, xlyem diameter, number of xlyem elements, and surface area of xylem) may have an affect on Mg uptake. Selection for LDR lines may alter all of above, as well as altering the plant's ability to extract water and nutrients from deeper levels in the soil profile. These factors in turn may alter the hypomagnesemic tetany potential of this species because Mg movement to plant roots is primarily by mass flow[1,7,9,11]. Therefore, the following experiment was conducted to characterize tall fescue roots and to study root anatomy effects on Mg influx under controlled conditions.

Materials and methods

Five clonal lines of tall fescue were used in this study: from breeding lines AU-5, AU-7, AU-264, AU-718, and a selection from Kentucky 31 (Ky 31). The clonal material was preconditioned in nutrient solution to produce propagules with roots free of soil contamination. Uniform single shoot propagules were removed from the 'parent' clones, washed for 2 hours in distilled water, and transferred into 12-liter tanks in the greenhouse.

Nutrient concentrations were: 0.25 mM KCl, 0.25 mM KH_2PO_4, 0.25 mM NH_4NO_3, 0.5 mM $CaCl_2$, 180 μM FeDTPA (diethylene triaminepentacetic acid), 46 μM B, 9 μM Mn, 0.8 μM Zn, 0.3 μM Cu, and 0.05 μM Mo. Magnesium concentrations of 3, 21, 42, 125, 250, and 500 μM were imposed on individual tanks by the addition of $MgSO_4$. The SO_4 concentration was adjusted with Na_2SO_4 to give a constant SO_4 concentration of 500 μM. Nutrient concentrations were monitored every 2 days by removing 50 ml of solution from each tank and determining the nutrient concentrations by standard methods. Nutrient concentrations were maintained by addition of nutrients as required and the concentrations did not vary more than 5% during the course of the experiment.

Solution pH was measured daily and maintained at 5.6 to 5.8 by adding HCl or NaOH. To minimize the fluctuation of the solution pH, the sodium salt of [2-(N-Morpholino) ethanesulfonic acid] (pH 6.15) was added to the nutrient solution for a final concentration of 1 mM. All tanks were vigorously aerated, and nutrient solutions were changed every 7 days. Temperature was maintained at 24° ± 5°C, and sunlight was supplemented with fluorescent light to produce a minimum of 250–300 $\mu Em^{-2} s^{-1}$ at the canopy for a 16-hour day.

An experimental unit consisted of two propagules (paired) of each clonal line at each Mg level. Individual propagules were supported by foam rubber collars in No. 6 plastic stoppers in a 0.5-cm-thick black plexiglass tank cover. After growing for 39 days, one propagule was harvested. The other propagule was harvested at 70 days. Roots were washed in diluted Ca solution (0.1 mM) for 15 minutes in an attempt to remove all Mg in the free space of root cells. The propagules were separated into shoots (leaf blades, leaf sheaths, and stems) and roots, freeze-dried, weighed, and ground to pass a 40-mesh screen. Root volume was determined at harvest using a water-displacement method. Concentrations of Mg in the tissue were determined by Inductively Coupled Argon Plasma (ICAP). Net influx rates (I_m) of Mg were calculated from the change in total Mg content and the change in fresh weight of tall fescue propagule roots using the following equation:

$$I_m = \frac{M_2 - M_1}{WR_2 - WR_1} \cdot \frac{\ln(WR_2/WR_1)}{t_2 - t_1}$$

where I_m is uptake rate per gram fresh weight of root, M is total elemental content in tall fescue propagule (leaves + roots), WR is fresh root weight, and t is time (days). I_m for the first growth period (subscripts 1 and 2) denotes initial and first harvest, and for the second growth period denotes initial and second harvest.

The kinetic constants K_m and V_{max} were calculated for each fescue line by plotting I_m/[Mg concentration]. These plots are called Eadie-Hofstee plots and they magnify departures from linearity which is not apparent in a double-reciprocal plot. The kinetic constant V_{max} is obtained by extrapolating to the Y axis (I_m) and the slope of the plot is $-K_m$.

The experimental design was a randomized complete block with three replications. Treatments were arranged in a factorial design (5 clones × 6 Mg concentrations). Data were analyzed using standard analysis of variance and regression analysis when differences due to Mg concentration were significant.

Roots for characterization were grown in the 12-liter tanks as previously described. Each tank contained 10 propagules of a single tall fescue clone. The experiment was replicated 10 times (50 tanks) with a completely randomized arrangement of the tanks in the greenhouse. The nutrient solution was the same as the standard solution, with 84 μM of Mg as $MgSO_4$. Twenty randomly selected roots were taken from each tank of propagules (a total of 200 roots) for characterization of each of the five clones. Roots removed were maintained in a petri dish with moist filter paper and root diameters were measured within 4 hours of removal from the clone. A hand-sectioned sample of 0.01 cm thickness was taken 7.5 cm behind the root apex, and examined under a light microscope. A scaled eyepiece was used to measure root and xylem diameters.

Results and discussion

Root anatomy

The 5 tall fescue lines were clustered into 3 groups on the basis of root diameter and xylem organization (Table 1). Tall fescue lines AU-7 and AU-264 had large diameter roots (LDR) with root diameters averaging 0.98 and 0.83 mm. Two lines (5 and 718, SDR) had root diameters averaging 0.72 mm. The Ky 31 clone root diameters averaged 0.69 mm. The reproducibility of the root diameter measurements was excellent, with CV's averaging less than 20%. At 39 days, root volume of the SDR lines (AU-5 and AU-718) were approximately double the root volume of the LDR (AU-7 and AU-264) (Table 2). However, at 70 days only the SDR line (AU-5) root volume was double the LDR lines. The larger root volume of the AU-5 line was a result of a greater number of secondary roots/cm of primary root than the other SDR line and both LDR lines.

Xylem organization was different for LDR's, SDR's, and Ky 31 (Fig. 1, Table 1). The LDR xylem elements were approximately equal in diameter and were arranged in a polyarch pattern. The average xylem

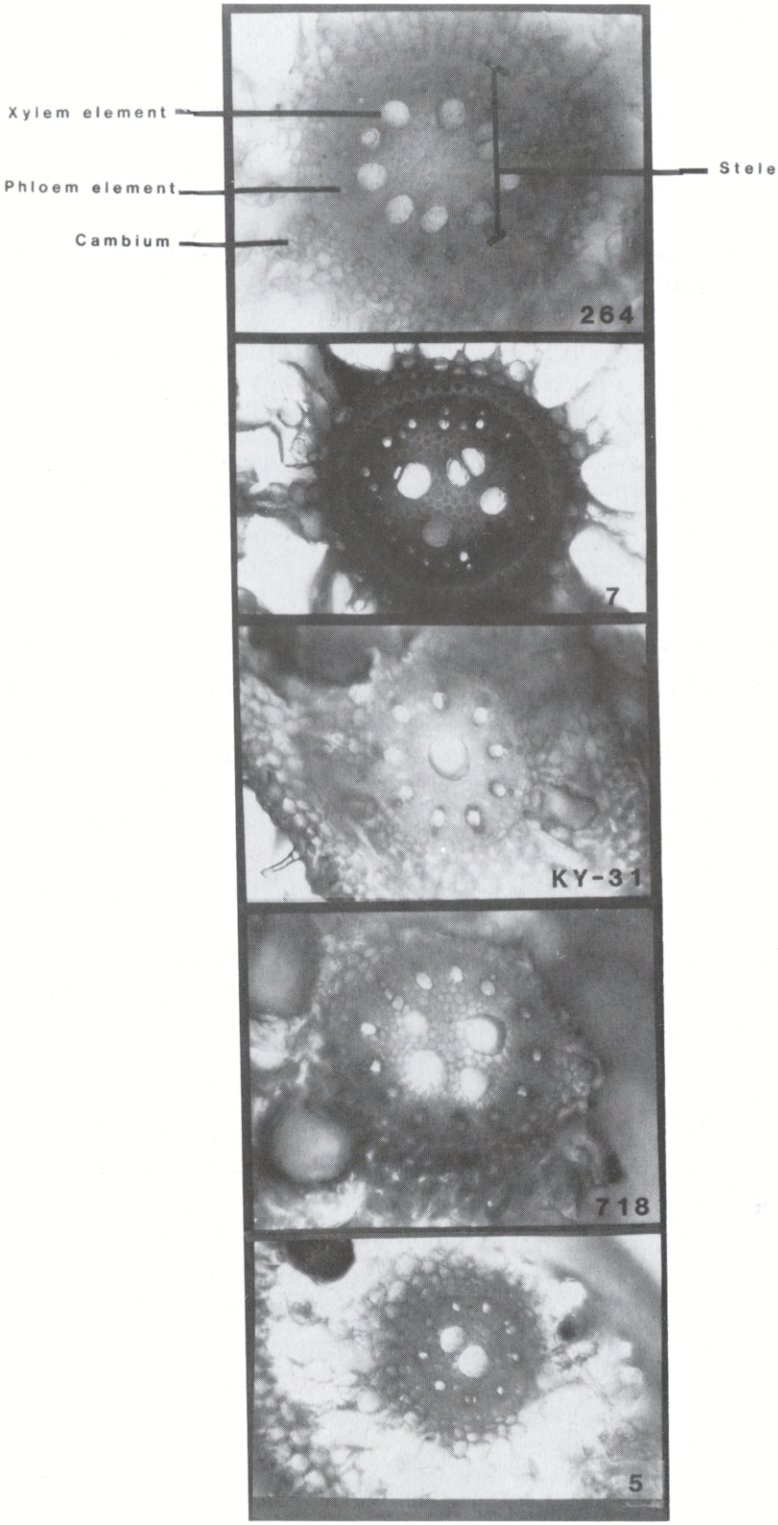

Xylem element
Phloem element
Cambium
Stele
264
7
KY-31
718
5

Table 1. Root morphology of tall fescue clones

Fescue lines	Root diameter (mm)		Xylem diameter (mm)
	Average	Range	Average
AU-7 (LDR)	0.98a[z]	1.04–0.92	0.18a
AU-264 (LDR)	0.83b	0.88–0.76	0.16a
AU-718 (SDR)	0.72c	0.76–0.66	0.14b
AU-5 (SDR)	0.72c	0.76–0.68	0.14b
Ky 31	0.69c	0.73–0.64	0.11c

[z] Fisher's protected LSD_{05} level.

diameters for LDR were 0.18 and 0.16 mm for lines AU-7 and AU-264, while the average xylem diameter for lines AU-5 and AU-718 was 0.14 mm and the single clone selection of Ky 31 was 0.11 mm. Xylem diameter of Ky 31 was smaller than either LDR or SDR lines and its organization was a monarch pattern of xylem arrangement and a relatively large xylem element in the center of the xylem.

Mg influx

Net influx rates of Mg were generally higher at all solution Mg concentrations in the first harvest period than in the second harvest period (Fig. 2). This supports the theory proposed by Elkins *et al.*[3] that the volume of root tissue without suberized endodermis is critical for Mg uptake, and that relative proportion of suberized endodermis increases with physiological age. Interactions between lines and harvest dates were significant at the 05 level of probability, and are shown graphically (Fig. 2). At harvest 2, both LDR lines exhibited lowest Mg uptake. However, at harvest 1, AU-264 was intermediate in uptake at low and medium solution Mg concentrations and highest in Mg influx rate at high solution Mg concentrations. Clearly, root morphology does affect Mg influx rate, but the effect is different as fescue roots mature.

Eadie-Hofstee constants

The K_m and V_{max} obtained from Eadie-Hofstee plots for AU-264 (Fig. 3) and a complete set of K_m and V_{max} for all five fescue lines are given in Table 3. At low Mg concentration for harvest 1, line AU-7 appears to have the lowest K_m for Mg influx. Although line AU-264 is classified as LDR, the K_m and V_{max} appear to parallel the SDR line AU-5. Lines AU-718 and Ky 31 appear to have similar mechanisms of uptake at low Mg concentrations in solution.

Fig. 1. Cross-section of mature roots of tall fescue lines AU-264, AU-7, Ky 31, AU-718 and AU-5 7.5 cm behind root apex.

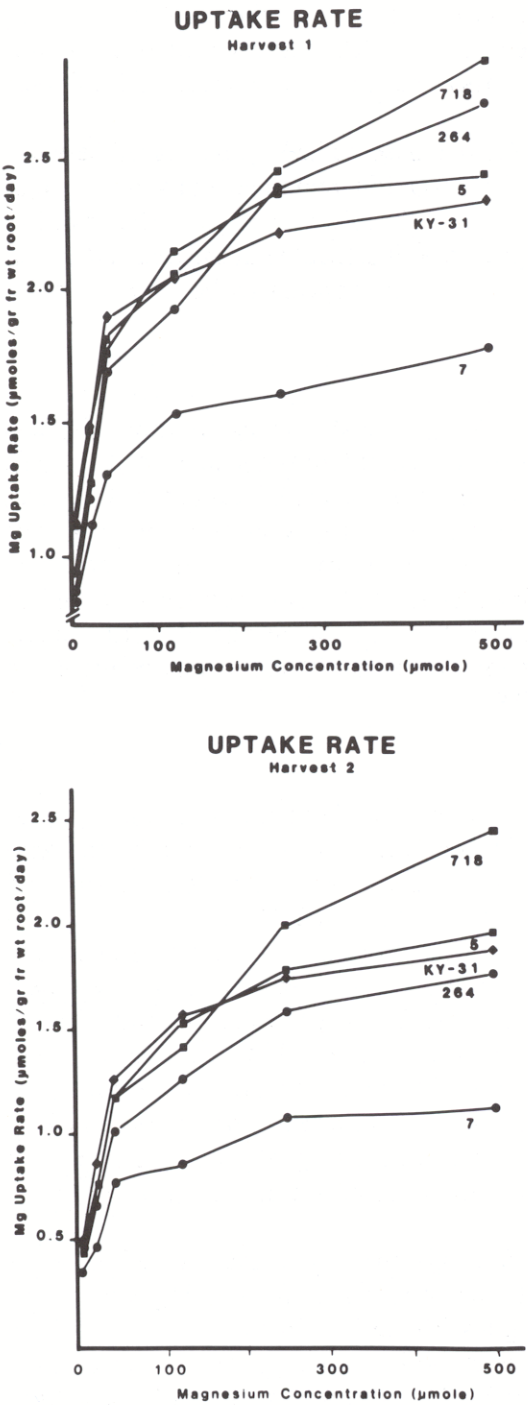


Fig. 2. Magnesium uptake for tall fesuce lines after 39 (harvest 1) or 70 (harvest 2) days of growth in nutrient solution with selected Mg concentration.

The low K_m of line AU-7 is also illustrated in the high solution Mg concentration range for harvest 1. Lines AU-5 and Ky 31 have similar K_m and V_{max}. Line AU-264 appears to have uptake characteristics similar to line AU-718.

For all but one fescue line, K_m increased while V_{max} decreased between harvest 1 and 2. The K_m of line AU-264 remained constant at high solution Mg between harvest 1 and 2. The low K_m for the low Mg concentration mechanism suggests that it is located at the plasmalemma, while the high concentration mechanism appears to be located at the tonoplast membrane[6].

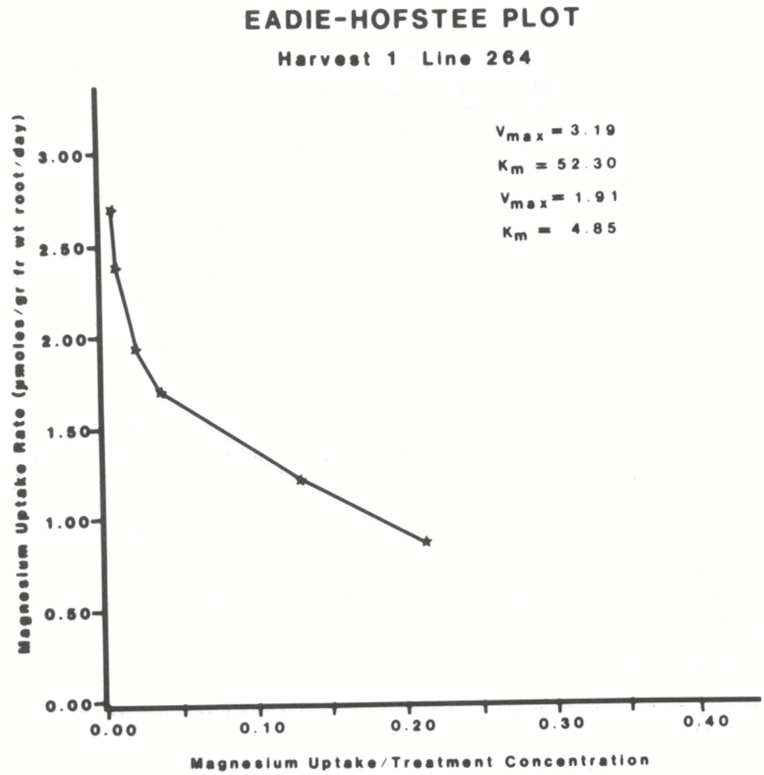
Leaf Mg concentrations

Clonal Mg concentrations in leaf tissue at varying solution Mg concentrations generally follow the curves and ranking described for Mg influx (Fig. 4). Therefore, Mg uptake may be a valid indicator of leaf Mg concentration and tetany potential. Leaf Mg concentration was not strongly responsive to solution Mg concentration changes at intermediate levels, especially for line AU-7. The LDR lines were lower in leaf Mg concentration at all solution Mg concentrations at both harvest dates. This suggests that Mg tetany potential in fescue may be increased by selection for lines with LDR.

Root Mg concentration

Root Mg concentration was quite variable at low solution Mg concentrations at harvest 1 (Fig. 3). Smooth quadratic curves for the relationship of root Mg concentration and solution Mg concentration were obtained with the more mature roots at harvest 2. However, the relationship between root diameters and tissue Mg concentration was altered in root tissue compared to leaf tissue. Lines AU-7 and AU-718 were lowest in root Mg concentrations at harvest 2, while lines AU-5 and AU-264 were highest. Root Mg concentration was much lower than leaf Mg concentration. Neither the lowered Mg concentration of the roots nor the lack of a consistent relationship of this character with root morphology is of practical importance when considering Mg tetany potential in fescue, since ruminants consume only the above-ground portion of the plant.

In summary, differences in root morphology of tall fescue lines were related to differences in Mg tissue concentrations and Mg influx rate, with LDR plants accumulating less tissue Mg. Physiological age of the tall fescue root was shown to affect the K_m and V_{max} with younger plants being more effective in Mg uptake. The root volume for tall fescue lines AU-5 and AU-718 was approximately 50% greater than lines AU-7 and


EADIE-HOFSTEE PLOT
Harvest 1 Line 264
Vmax = 3.19
Km = 52.30
Vmax = 1.91
Km = 4.85
Magnesium Uptake Rate (µmoles/gr fr wt root/day)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.00
0.10
0.20
0.30
0.40
Magnesium Uptake/Treatment Concentration

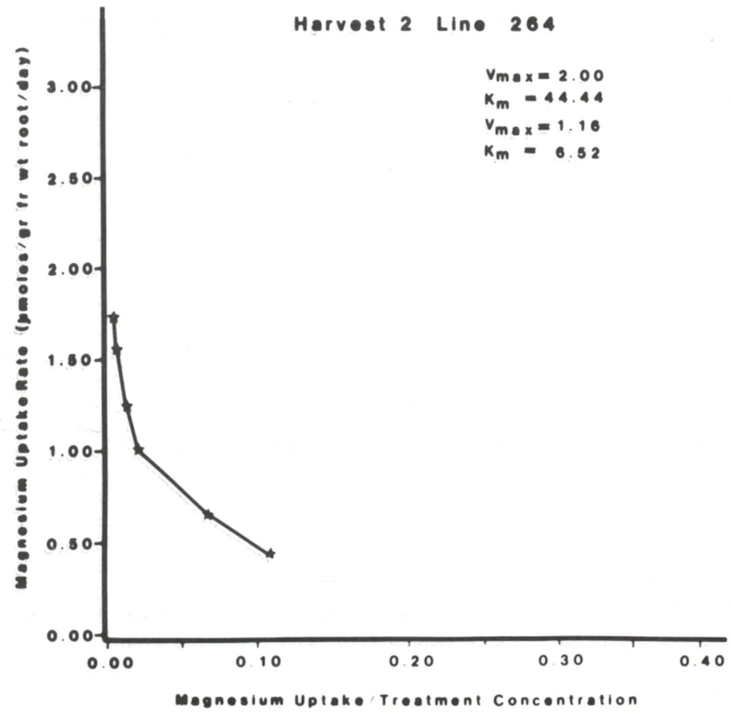

Harvest 2 Line 264
Vmax = 2.00
Km = 44.44
Vmax = 1.16
Km = 6.52
Magnesium Uptake Rate (µmoles/gr fr wt root/day)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.00
0.10
0.20
0.30
0.40
Magnesium Uptake/Treatment Concentration

Table 2. Root volume (cm^3) of tall fescue lines after 39 or 70 days of growth in nutrient solution containing selected Mg concentrations

Fescue lines	Mg concentration (μM)						line mean
	3	21	42	125	250	500	
39 days							
AU-7 (LDR)	7.3	7.0	8.3	4.3	7.3	7.3	7.0
AU-264 (LDR)	8.0	7.3	5.6	7.3	5.3	6.0	6.6
AU-5 (SDR)	16.3	15.0	14.3	12.3	14.6	19.0	15.3
AU-718 (SDR)	18.6	16.3	12.7	10.0	10.6	10.0	13.0
Ky 31	11.7	14.3	10.3	8.6	10.7	10.0	10.9
Concentration means	12.4	12.1	10.3	8.5	9.7	10.4	
70 days							
AU-7 (LDR)	31.6	31.0	17.6	24.3	17.0	30.6	25.4
AU-264 (LDR)	36.3	29.0	21.3	31.0	28.3	22.6	28.1
AU-5 (SDR)	40.6	54.0	61.3	41.0	46.0	62.6	50.9
AU-718 (SDR)	49.0	43.3	31.3	32.6	33.3	45.6	39.2
Ky 31	42.3	33.0	47.3	31.6	34.3	29.6	36.4
Concentration means	40.00	38.06	35.8	32.1	31.8	38.3	

39 days $FLSD_{05}$ lines = 2.41
39 days $FLSD_{05}$ lines = 2.64
70 days $FLSD_{05}$ lines = 8.65
70 days $FLSD_{05}$ lines = NS

Table 3. K_m and V_{max} for Mg uptake for tall fescue lines grown in selected Mg concentrations for 39 or 70 days

Fescue lines	Low concentration		High concentration	
	K_m[z]	V_{max}[y]	K_m	V_{max}
39 days				
AU-7 (LDR)	2.86	1.46	15.25	1.86
AU-264 (LDR)	4.85	1.91	52.30	3.19
AU-5 (SDR)	4.64	1.97	20.38	2.65
AU-718 (SDR)	3.11	1.97	49.24	3.25
KY 31	3.15	2.06	13.77	2.41
70 days				
AU-7 (LDR)	6.85	0.89	34.21	1.30
AU-264 (LDR)	6.52	1.16	44.44	2.06
AU-5 (SDR)	9.22	1.41	39.46	2.21
AU-718 (SDR)	7.03	1.35	90.00	3.06
KY 31	8.15	1.50	26.62	2.05

[z] K_m — μMoles
[y] V_{max} — μMoles/g fr root wt/day

Fig. 3. A Eadie-Hofstee plot of tall fescue line 264 to obtain K_m and V_{max} for 39 or 70 day of growth in nutrient solution containing selected Mg Concentration.

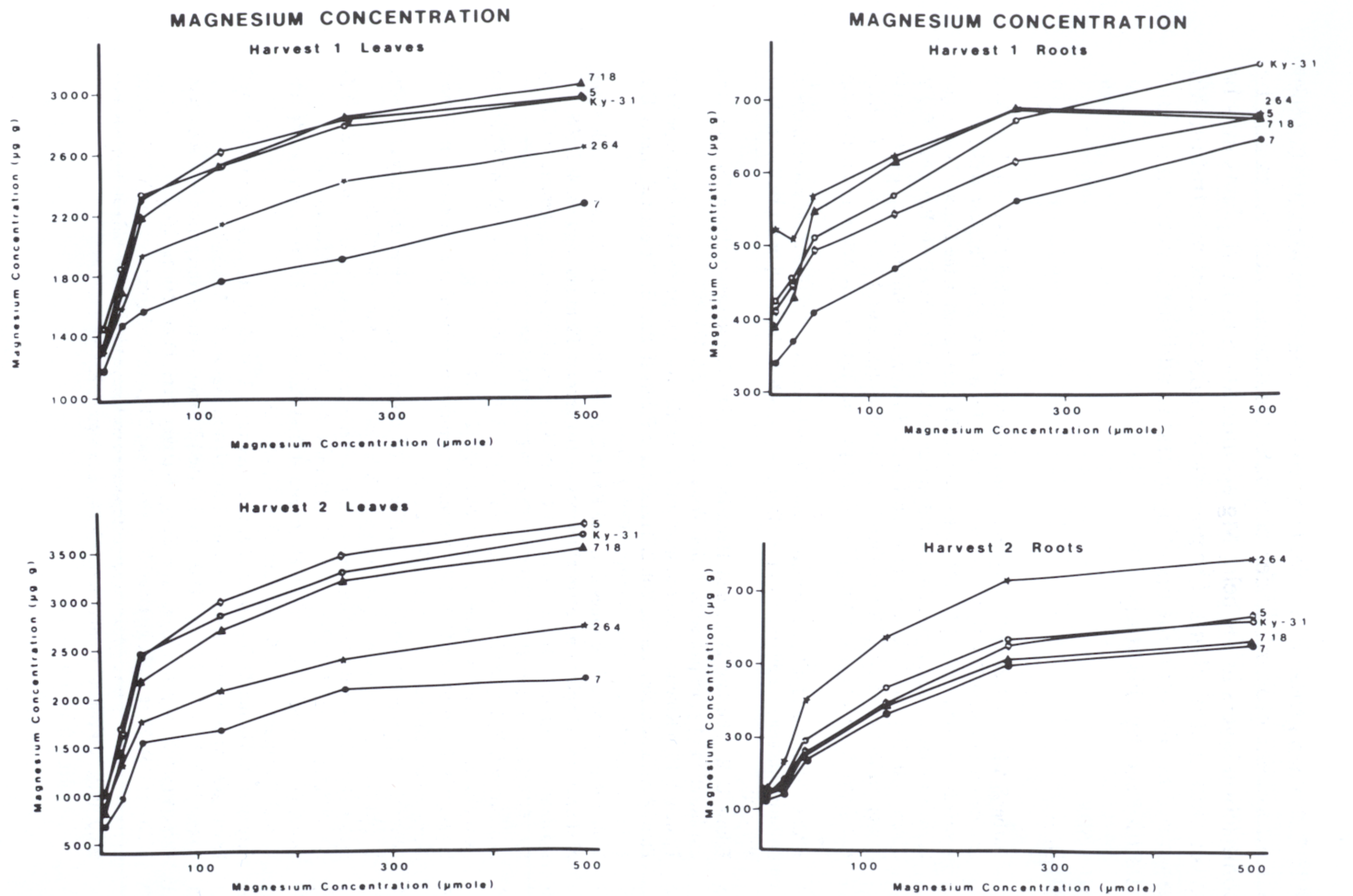


Fig. 4. Leaf and root Mg concentration for tall fescue lines after 39 (harvest 1) or 70 (harvest 2) days of growth in nutrient solution containing selected Mg concentrations.

AU-264 when grown in nutrient solutions. If these relationships hold for a root system growing under confined conditions, such as caused by compaction layers in the field, Mg accumulation in LDR plants may be lower than in SDR plants. The possible benefits of LDR penetration into lower soil profiles and the resulting access to additional nutrients cannot be addressed by this study.

References

1 Barber S A 1974 Influence of plant root on ion movement in soil. pp 525–564 *In* The Plant Root and its Environment. Ed. E W Carsun. University Press of Virgina, Charlottesville, Va.

2 Edwards J H and Pedersen J F 1985 Why won't tall fescue persist in the Coastal Plain region of the Southeast? Proc. Amer. Forage and Grassland Cong., Hershey Park, Penn. pp 129–136.

3 Elkins C B, Haaland R L, and Hoveland C S 1983 Biological availability of magnesium to plants. pp 55–60 *In* Role of Magnesium in Animal Nutrition. Eds. J P Fontenot, G E Bunce, K E Weeb, Jr., and Vivien G Allen. Virginia Polytechnic Institute and State Univ., Blacksburg, Va.

4 Elkins C B, Haaland R L, Rodriguez-Kabana R, and Hoveland C S 1979 Plant-parasitic effects on waste use and nutrient uptake of small-and large-rooted tall fescue genotypes. Agron. J. 71, 497–500.

5 Elkins C B, Thurlow D L, and Hendrick J G 1983 Conservation tillage for long-term amelioration of plow pan soils. J. Soil Water Conserv. 38, 305–307.

6 Epstein E 1976 Kinetics of ion transport and the carrier concept. pp 70–94 *In* Encyclopedia of Plant Physiology, New series, Vol. 2, Transport in Plants II. Part B. Tissues and organs. Eds. U Luttge and M G Pitman. Springer-Verlag, Berlin.

7 Follett R F, Power J F, Grunes D L and Kleen C A 1977 Effect of N, K, and P fertilization, N source, and clipping on potential tetancy of bromegrass. Plant and Soil 48, 485–508.

8 Haaland R L, Elkins C B and Hoveland C S 1978 A method for detecting genetic variability for grass tetany potential in tall fescue. Crop Sci. 18, 339–340.

9 Hannaway D B, Leggett J E, Bush L P, and Shuler P E 1984 Magnesium (Mg) and Rubidium (Rb) absorption by tall fescue. J. Plant Nut. 7, 1127–1147.

10 Nguyen H T and Sleper D A 1981 Genetic variability of mineral concentration in *Festuca arundinacea* Schreb. Theor. Appl. Genetics 59, 57–63.

11 Oliver S and Barber S A 1966 An evaluation of the mechanism governing the supply of Ca Mg, K and Na to soybean roots (*Glycine max*.) Soil Sci Soc Am. Proc. 30, 82–86.

12 Sleper D A 1979 Plant breeding, selection, and species in relation to grass tetany. pp 63–77 *In* Grass Tetany. Eds. V V Rendig and D L Grunes. Am. Soc. Agron., Madison, Wis.

13 Sleper D A, Garner G B, Asay K H, Boland R, and Pickett E E 1977 Breeding for Mg, Ca, K, and P content in tall fescue. Crop Sci. 17, 433–438.

14 Sleper D A, Garner G B, Nelson C J, and Sebaugh J L 1980 Mineral concentration of tall fescue genotypes grown under controlled conditions. Agron. J. 72, 720–722.

15 Tobert H A, Edwards J H, and Pedersen J F 1985 Relative nutrient absorption by fescue lines. Proc. Amer. Forage and Grassland Cong., Hershey Park, Penn. pp 259–265.

16 Williams C B, Elkins C B, Haaland R L, Hoveland C S, and Rodriguez Kabana R 1981 Effects of root diameter, nematodes and soil compaction on forage yield of two tall fescue genotypes. Proc. Inter. Grassland Congr. June 15–24, Lexington, Ky. pp 121–124.